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Abstract

Models of addiction and addiction memory propose that drug-associated cues elicit incentive effects in drug users, which play an important role in maintenance of drug use and relapse. Incentive effects have been demonstrated for smoking and alcohol-related cues but evidence for heroin-related cues has been inconclusive. Furthermore, it is unknown whether appetitive effects of heroin-related cues persist after prolonged abstinence, although heroin addiction is known to have high relapse rates. Therefore, we investigated implicit and explicit valence of heroin-related cues in dependent users at different stages of abstinence using affective startle modulation. In *Study I*, 15 current heroin users were measured before and after detoxification. Correspondingly, 15 healthy control participants were tested twice at an interval of 14 days. In *Study II,* 14 long-term abstinent heroin users were additionally measured in a single session. Implicit processing of drug-related stimuli was assessed using affective startle modulation by pictures of heroin and smoking scenes. Explicit reactions were measured using ratings of valence and craving. In contrast to controls, heroin-dependent participants showed a clear reduction of startle response during heroin-related pictures ($p<0.05$). Detoxification did not significantly change their startle responses to heroin-cues. No difference between non-detoxified current and long-term abstinent heroin users was found in implicit reactions to heroin-cues, whereas explicit measures differed between both groups (all $p<0.05$). After detoxification and even after prolonged abstinence, heroin cues still exert implicit appetitive effects in heroin users. This implies that drug-induced adaptations of reward circuits are long-lasting, resulting in a highly stable addiction memory.

Key words: abstinence**,** addiction memory, affective startle modulation, cue-reactivity, detoxification, heroin

Introduction

Drug dependence, opiate dependence in particular, can be considered as a chronic relapsing disorder (Hser et al., 2001). Models of addiction propose that cue reactivity (i.e. elicitation of conditioned responses on psychological, physiological, and behavioral levels by drug-associated stimuli) is of particular importance in the maintenance of drug use and relapse (Carter and Tiffany, 1999; Fatseas et al., 2011). Traditional theories of drug cues hold that drug-associated stimuli evoke conditioned reactions that trigger withdrawal-like and aversive responses (Koob et al., 1997; Wikler, 1973). More recently, there is evidence that drug-associated cues may rather be processed as appetitive leading to the same behavioral and neurobiological responses like the drug itself (Dempsey et al., 2007; Geier et al., 2000; Mucha et al., 2000). Moreover, the incentive-sensitization theory proposes that drug consumption produces incremental neuroadaptations in the mesolimbic dopamine pathways, rendering them hypersensitive to drugs and drug-associated stimuli (Robinson and Berridge, 2000). This sensitization turns the act of drug taking and stimuli associated with it into powerful incentives that produce a strong feeling of "craving" for the preferred drug. Craving commonly refers to the subjective experience of wanting to use a drug (Tiffany and Wray, 2012) and the urge to re-experience the effect of a psychoactive substance (UNDCP/WHO, 1992).

This is in line with multiple theories of addiction memory, which propose shared mechanisms between memory, learning, and addiction (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman et al., 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996). Repeated pairings of drugs and environment are supposed to produce long-term, maybe permanent, neuroadaptive effects in motivational networks that lead to the establishment of compulsive drug-seeking habits (Kelley, 2004; Robbins and Everitt, 2002). These associations and the craving elicited by drug-related stimuli might be in part unconscious (Tiffany, 1990). Drug

memories are therefore supposed to be long-lasting and implicit. This assumption is consistent with high relapse rates among heroin users following treatment (O'Brien, 1997).

A well-established non-subjective method to measure the implicit valence of drug-associated cues is the affective startle modulation (Geier et al., 2000; Rehme et al., 2009). The acoustic startle reflex (ASR) is a solid marker of emotional reactivity (Lang et al., 1990) and is elicited by unexpected, intense environmental stimuli such as a sudden, loud noise. The affect-modulated startle methodology has been repeatedly used in drug research by including photographs of drug paraphernalia or drug intake rituals and measuring the suppression of the startle response by drug-related scenes relative to neutral scenes (referred to as cue-related startle suppression, CSS). In general, these studies confirmed incentive theories because they consistently reported CSS in addicted users of alcohol or nicotine while viewing drug-related cues (Cinciripini et al., 2006; Dempsey et al., 2007; Geier et al., 2000; Mucha et al., 2000). Enhanced attentional processing of heroin cues and cue-induced reactivity have been demonstrated for heroin users with other methods, like Stroop tasks and electrodermal responses (Franken et al., 2000; Lubman et al., 2008), and some of these found that cue reactivity might be a predictor for future heroin use and relapse after treatment (Fatseas et al., 2011; Lubman et al., 2009; Marissen et al., 2006). However, the implicit valence of heroin cues before and after detoxification and withdrawal therapy was not investigated so far. Furthermore, it is unknown whether heroin cues elicit appetitive effects in long-term heroin-abstinent individuals. However, sustained appetitive value of drug-related cues might be a driving force underlying the high relapse rates in heroin addiction (Carter and Tiffany, 1999; Fatseas et al., 2011).

Hence, we conducted two studies investigating the implicit valence of drug-related stimuli in different states of abstinence. In Study I, we analyzed the response to heroin-related cues in current heroin-dependent participants before and after a two week detoxification program, as well as in control participants tested within the same time interval. Smoking cues were used

because of the common co-use of nicotine in heroin-dependent patients. For the same reason, for the control group only smokers were recruited. ASR was evoked during the presentation of positive, negative, and neutral pictures from the *International Affective Picture System* (IAPS)(Lang et al., 1997) and drug-related cues in order to assess the implicit valence of stimuli. The subjective evaluation of the stimuli was measured by ratings of valence and craving. According to sensitization and addiction memory theories, we hypothesized that heroin cues have appetitive effects in heroin-dependent participants before and after detoxification.

The aim of Study II was to investigate the influence of successful long-term abstinence on affective processing of heroin-associated cues. Because of the significance of drug-related cues in relapse (Carter and Tiffany, 1999; Fatseas et al., 2011), it is important to investigate whether a history of opiate use is associated with persistent implicit memories and enhanced cue-reactivity or if processing changes after successful abstinence. Therefore, a group of long-term abstinent heroin users was compared with current heroin users before detoxification and controls. Here, we expected long-term abstinent heroin-dependent participants to be more similar to current opiate users than to controls in their implicit reactivity towards heroin cues, as the incentive sensitization theory and addiction memory models posit memory traces to be persistent.

Experimental procedures

Participants

Study I was carried out with two groups: 15 currently heroin-dependent participants and 15 healthy participants with no history of opiate use (Table 1). Heroin-dependent participants were enrolled in a residential detoxification program at the Department of Psychiatry of the University of Bonn, Germany. Intravenous heroin dependence was diagnosed following the Diagnostic and Statistical Manual-IV (DSM-IV) criteria (American Psychiatric Association, 1994). Detoxification therapy was standardized by treatment with methadone (up to 20mg/day), which was decreased within 8-12 days of inpatient treatment. None of the patients in the detoxification program received methadone maintenance treatment prior to detoxification. The control group was recruited from the general population by advertisement.

Study II compared a non-overlapping sample of 14 long-term abstinent heroin users (assessed once) with the 15 heroin-dependent participants (before detoxification) and 15 control participants (first measurement) from Study I. Long-term abstinent heroin users had to be diagnosed previously with intravenous heroin dependence according to DSM-IV criteria. They were recruited from a study on genetic influences on vulnerability to heroin dependence (Xu et al., 2004). In conjunction with a five-year follow up, only subjects who were abstinent for at least one year were considered for the present study (mean abstinence duration (SD)=18.93 (±20.45) months). They did not receive methadone or heroin maintenance therapy. Abstinence was confirmed by urine toxicology.

All participants were active smokers. Exclusion criteria comprised psychiatric or neurological disorders, and substance use disorders other than nicotine and opioid dependence for heroin users, use of psychotropic medication, and medical conditions concerning eyes, ears and equilibrium organs.

The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn and was carried out in accordance with the declaration of Helsinki. After receiving a written and oral description of the aim of this study, all participants provided written informed-consent statements and received either 50€ (current heroin users and controls) or 25€ (former heroin users) for their participation.

Experimental procedures

For Study I heroin-dependent participants were first tested when they started detoxification therapy (T1) and again when they finished therapy after 14 days of treatment (T2). Accordingly, control subjects were also tested on two experimental days with an interval of 14 days. In Study II, long-term abstinent heroin users were tested once and compared to heroin-dependent participants and controls at T1. Before the experiment, abstinent heroin users had to provide a urine sample to test for abstinence from opiates and other illegal drugs. All participants were asked for their drug history, daily cigarette consumption, momentary desire for heroin and momentary withdrawal symptoms by means of self-designed questionnaires. The questionnaire for withdrawal symptoms included 23 symptoms according to DSM-IV criteria. Their occurrence had to be rated on a 5-point scale. Further, the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-A) (Lehrl, 1999), a standardized German vocabulary test, was carried out for the estimation of verbal intelligence quotient (IQ). The Hopkins Symptom Checklist (SCL-90-R) (Franke, 1995) was employed as a self-report psychological status symptom inventory. Before the startle procedure, all participants underwent a hearing screening to insure hearing within normal limits. Participants would have been excluded on the basis of hearing impairment at 40dB (1000Hz). Participants had to abstain from smoking for at least 60min prior to the study, as the acute effects of cigarette smoking have been shown to influence startle amplitudes (Kumari et al., 1996). The startle

experiment was carried out in a calm and darkened room. Subjects were instructed to view the pictures and to ignore any noises from the headphones. After the startle procedure, all subjects viewed each picture individually and rated the perceived valence of the picture on 10-point scales (i.e., "How do you perceive the present picture?", 0=very unpleasant, 10=very pleasant), as well as their craving for nicotine and heroin (i.e., "How much desire to smoke/to take heroin does the present picture induce?", 0=no desire, 10=strong desire).

Stimulus material and presentation

Stimulus material comprised 56 colored photographs, sixteen pictures showing the beginning and end of a heroin injection scene (**Supplemental Material Figure S1**), and 16 pictures showing the beginning and end of smoking a cigarette, which were taken from a previous study (Mucha et al., 1999, Experiment 2)(**Supplemental Material Figure S2**). Drug-associated stimuli depicted the beginning and end scenes of drug use because begin- and end-pictures of smoking and alcohol consumption scenes were shown to be processed differently (Mucha et al., 2008; Nees et al., 2011; Stippekohl et al., 2010). Twenty-four control pictures comprised eight pleasant, neutral and negative scenes or objects, respectively. The task was presented using the Experimental Run Time System (ERTS) software (Berisoft Cooperation, Frankfurt, Germany). Details on the stimulus material are given in the **Supplementary text.**

Pictures were arranged in two blocks of 28 photographs in a fixed-randomized and balanced order. Each block started with a 4-min habituation period of 70dB background white noise. The pictures were presented for 7 to 8s, followed by a black monitor lasting for 16.5 to 25.5s. During 6 out of 8 pictures (75%) of each category an acoustic startle response (ASR) was evoked, resulting in 40 ASR trials. The startle probe consisted of a burst of white noise with an intensity of 116dB (duration 40ms, instantaneous rise/fall time, bandwidth approximately 300 Hz-18 kHz) presented binaurally using headphones (TDH-39-P; Maico). Startle probes were presented 2.5, 4.5, and 5.5s after picture onset.

Data recording and reduction

ASR was recorded and analyzed as described previously (Rehme et al., 2009). In brief, the eye-blink component of the ASR was measured by an electromyographic (EMG) startle system (EMG-SR-Lab; San Diego Instruments, Inc., San Diego, CA). EMG activity was measured from the right *orbicularis oculi* muscle using two silver/silver chloride electrodes. A reference electrode was placed on the glabella. Detailed recording settings and data reduction procedures are given in the **Supplementary text**.

Data analysis

Demographic variables, smoking and heroin using behavior and craving measures were analyzed using analyses of variance (ANOVAs). Differences in sex distribution between groups were tested using χ^2-tests.

The individual startle amplitudes were standardized according to the individual mean and the standard deviation of the startle amplitudes of the control scenes. Standardization was appropriate as the raw startle magnitude showed strong variation across subjects (Patrick et al., 1993). Age was introduced as a covariate because it has been shown to influence affective startle modulation (Feng et al., 2011). Therefore, the standardized startle amplitudes were analyzed parametrically by mixed-design ANCOVAs controlling for age. The difference between the standardized amplitude during neutral scenes minus the standardized amplitude during drug-related scenes was used as an index for the CSS. Details on the data analyses are given in the **Supplementary text**. ANCOVAs were followed by Bonferroni-corrected pair-wise comparisons and simple main effects analyses. Statistical tests were considered significant at a level of $p<0.05$ (two-tailed). Analyses were performed using the PASW Statistics 18 for Windows.

Results

Study I: Current heroin-dependent participants before and after detoxification therapy

Demographic characteristics

Healthy control subjects and heroin-dependent participants did not differ in verbal IQ, age, cigarettes smoked per day (CPD) or sex distribution. Heroin-dependent participants had less years of education ($F(1,29)=8.56$, $p=0.007$) and, as expected, scored higher on the SCL-90-R sum score ($F(1,29)=9.23$, $p=0.005$). Sociodemographic characteristics and drug use parameters are presented in **Table 1 and 2**.

Startle modulation by negative, neutral and positive standardized stimuli

Raw startle magnitudes did not differ significantly between groups on first (T1) and second (T2) assessment (all $p>0.250$), although heroin users showed slightly decreased startle reactivity (**Supplemental Material Figure S3).** Negative, neutral and positive pictures from the IAPS induced a linear startle pattern ($F(1,28)=19.83$, $p<0.001$) in T1 and T2 (**Figure 1A and 1B**). The mixed-design ANCOVA yielded no group, time or picture category main effects (all $p>0.220$) and no significant interactions (all $p>0.255$). A priori predicted pair-wise comparisons revealed significant differences between negative and positive stimuli and positive and neutral stimuli across groups (all $p<0.001$).

Drug cue effects

A Mixed-design ANCOVA revealed a significant time*category interaction ($F(3,81)=3.17$, $p=0.030$) and category*group interaction ($F(3,81)=2.73$, $p=0.050$). Heroin-dependent participants at T1 showed a significant CSS during heroin end ($F(1,28)=5.78$, $p=0.023$) and smoking end pictures ($F(1,28)=10.10$, $p=0.004$). In control participants, a significant CSS was found during smoking end pictures ($F(1,28)=12.07$, $p=0.002$). A significant difference

between T1 and T2 was found for smoking end pictures in heroin-dependent participants ($F(1,28)=9.84$, $p=0.004$). In control participants, no significant differences were found between T1 and T2 (all $p>0.268$)(**Figure 1C and 1D**).

Explicit picture rating (valence)

A significant main effect for picture category on valence ratings was found for the control pictures ($F(2,56)=120.94$, $p<0.001$.) Both groups rated the IAPS pictures according to their assumed valence (all paired comparisons $p<0.001$) at T1 and T2 (data not shown).

For drug-associated pictures, a significant main effect for category ($F(3,81)=34.12$, $p<0.001$) and significant interactions for category*group ($F(3,81)=5.90$, $p=0.001$) and time*group ($F(3,81)=4.94$, $p=0.035$) were found. At T1 and T2, heroin-dependent participants rated heroin begin and end cues significantly more positive than control participants (all $p<0.010$). No significant group difference was found for smoking pictures (all $p>0.081$).

Furthermore, heroin-dependent participants rated heroin end pictures ($F(1,28)=5.13$, $p=0.032$) and smoking begin pictures ($F(1,28)=11.68$, $p=0.002$) significantly more pleasant after therapy than before, whereas control participants showed no change in their valence ratings between T1 and T2 (all $p>0.468$)(**Figure 2A and 2B**).

Explicit picture rating (craving)

With regard to the ratings of craving for heroin, a significant main effect for group ($F(1,25)=18.54$, $p<0.001$) and category ($F(3,75)=15.84$, $p<0.001$) and a significant interaction for category*group ($F(3,75)=15.91$, $p<0.001$) were found. At T1, heroin-dependent participants reported more craving than controls for heroin begin ($F(1,25)=11.410$, $p=0.002$) and heroin end pictures ($F(1,25)=13.53$, $p=0.001$), while no significant difference was found for smoking cues. There was no significant difference in craving ratings between T1 and T2 (all $p>0.093$). At T2, heroin-dependent participants reported more craving for heroin than

control subjects during all drug-related picture categories (heroin begin: F(1,25)=12.82, p=0.002; heroin end: F(1,25)=11.50, p=0.002; smoking begin: F(1,25)=4.23, p=0.050; smoking end: F(1,25)=4.91, p=0.036)(**Figure 3A and 3B**). Craving to smoke cigarettes differed neither between groups nor time points (**Supplemental Material Figure S4A and S4B**).

Study II: Long-term abstinent heroin users

Demographic characteristics

Abstinent heroin users, heroin-dependent participants and control participants showed a comparable sex distribution ($\chi^2_{(2)}$=3.75, p=0.153) and did not differ in CPD (F(2,42)=3.68, p=0.537). However, groups differed in age, years of education, IQ, and SCL-90-R sum score (all p<0.045). Abstinent heroin users and heroin-dependent participants differed in the heroin withdrawal score (F(1,28)=8.13, p=0.008)(**Table 1 and 2**).

Startle modulation

For IAPS control pictures, the mixed-design ANCOVA yielded neither a significant main effect nor an interaction (all p>0.074). As a priori predicted, pair-wise comparisons revealed significant startle magnitude differences between negative and neutral stimuli (p<0.001) as well as between negative and positive pictures (p<0.001)(**Figure 4A**).

Comparing startle magnitudes during drug-related cues between controls and abstinent heroin users, a 4*2 (category*group) mixed-design ANCOVA showed a significant main effect for group (F(1,26)=5.45, p=0.028). There was no significant difference between abstinent heroin users and current heroin-dependent participants (F(1,26)=0.68, p=0.419). Former heroin users showed a significant CSS for heroin begin and heroin end pictures (all p<0.050)(**Figure 4B**). Raw startle magnitude was slightly smaller in current heroin users, but did not differ significantly between groups (F(2,43)=1.12, p=0.335)(**Supplemental Material Figure S5**).

Explicit picture rating (valence)

For the IAPS pictures, there was a significant main effect of picture category ($F(2,82)=66.13$, $p<0.001$). The pictures were rated by all three groups according to their assumed valence (all paired comparisons $p<0.001$)(data not shown).

For drug-associated pictures, there was a significant main effect for picture category ($F(3,123)=43.50$, $p<0.001$) and a significant interaction of category*group ($F(6,123)=6.53$, $p<0.001$). Abstinent heroin users rated heroin begin ($p=0.022$) and heroin end ($p=0.028$) significantly less pleasant than current heroin-dependent participants. No difference could be found between abstinent heroin users and controls in their valence rating of heroin-associated pictures (all $p>0.427$). Ratings for smoking pictures were not different between heroin-dependent participants and abstinent heroin users (all $p>0.705$). Abstinent heroin users rated smoking begin cues ($p=0.050$) and smoking end pictures ($p=0.033$) significantly less pleasant than control participants (**Figure 5A**).

Explicit picture rating (craving)

The analysis of ratings of craving for heroin revealed a significant main effect for group ($F(2,38)=10.21$, $p<0.001$) and category ($F(3,114)=12.38$, $p<0.001$) and a significant interaction of category*group $F(6,114)=7.26$, $p<0.001$). For heroin begin and end pictures, abstinent heroin users showed significantly less craving than current heroin-dependent participants ($p=0.014$, $p=0.004$). There was no difference in craving between controls and abstinent heroin users (all $p>0.147$). For smoking pictures, no group differences were found (all $p>0.170$)(**Figure 5B**). Ratings of craving to smoke showed no significant group differences (**Supplemental Material Figure S6**).

Discussion

The present study assesses the implicit and explicit reactivity to heroin-related cues before and after detoxification therapy and after long-term heroin abstinence. Heroin-dependent patients show normally modulated affective reactivity to emotional control pictures suggesting that the overall emotional reactivity is not impaired, which is in line with Walter et al. (2011), who also failed to find differences in startle reactivity to emotional pictures. However, results are inconsistent with Lubman et al. (2008; 2009) reporting reduced responsiveness to natural reinforcers in heroin users previously. Heroin-dependent participants, in contrast to controls, show a significant reduction in startle response during stimuli depicting the end of a heroin injection scene compared to neutral stimuli. Valence ratings confirm that heroin users perceive heroin pictures as more pleasant than control participants, whereas both groups show no difference in valence ratings of smoking pictures. This is in line with incentive theories of addiction proposing that drug associated cues are processed as appetitive or motivational incentives (Robinson and Berridge, 2000; Stewart, 1983; Wise, 1988). Incentive theories of drug addiction have been confirmed for other drugs, e.g., nicotine (Dempsey et al., 2007; Geier et al., 2000; Rehme et al., 2009) and alcohol (Grüsser et al., 2002; Heinz et al., 2003; Mucha et al., 2000), but results for heroin cues were undetermined so far. Walter et al. (2011) did not find a difference in startle response between neutral and drug-related stimuli, but opposed to our study, they used less specific and standardized drug pictures. Moreover, their patients were recruited from an opioid-maintained treatment program and were treated with stable doses of methadone or heroin. Thus, opioid-maintanance might damp the emotional reactivity of heroin-dependent patients (Walter et al., 2011). Our finding that heroin-cues are not perceived as aversive but serve as emotional and maybe motivational incentives is also in line with recent findings suggesting enhanced activation of reward-related brain areas (e.g., the anterior cingulate cortex and basal ganglia) in response to heroin-related cues in heroin users (Wang et al., 2011).

One novel finding is that the incentive value of heroin-related cues for heroin users persists after a 14-day detoxification therapy. Interestingly, explicit valence ratings imply that heroin end and smoking begin pictures are experienced as more pleasant after detoxification than before. These results are consistent with the finding that alcohol-related stimuli have appetitive qualities during alcohol detoxification and early abstinence (Grüsser et al., 2002). In addition, it has been shown that the reward system remains responsive to heroin-related cues in methadone maintenance patients with a history of heroin use (Langleben et al., 2008) and recent abstinence (Daglish et al., 2001). Nevertheless, craving in response to heroin-related cues did not change after detoxification therapy. This finding is in line with previous studies proposing a protracted abstinence syndrome even after longer drug free periods (Fatseas et al., 2011; Shi et al., 2007). Thus, strong appetitive effects of drug-related cues may support relapses after successful detoxification.

Another novel finding of the present study is that implicit incentive effects can still be measured even after at least one year of abstinence. Former heroin users do not differ from current heroin users, both groups showing suppressed startle responses to heroin-related stimuli. Recently, Shi et al. (2008) found a persistent decrease in the brain dopamine transporter (DAT) in the striatum of prolonged abstinent heroin abusers. Together, these findings support theories of an addiction memory, possibly based on long-lasting, or even permanent alterations in the dopamine system, which increases the vulnerability to relapse even after long-term abstinence (Kalivas and Volkow, 2005; Kelley, 2004). In sum, these findings corroborate the close association between reward-related learning, memory, and addiction postulated by theories of addiction and addiction memory (Berke and Hyman, 2000; Hyman and Malenka, 2001; Hyman et al., 2006; Kelley, 2004; Robbins and Everitt, 2002; White, 1996).

Notably, the implicit appetitive reactions of former heroin users to heroin-related stimuli are in contrast with their explicit measures: Valence and craving ratings of heroin-related stimuli

did not differ between former heroin users and controls. This finding supports the hypothesis that explicit and implicit processing of drug-related cues might be dissimilar (Grüsser et al., 2002; Tiffany, 1990). Even though sensitization of neural pathways seems to be persistent, former heroin users are obviously able to maintain abstinence. Top-down controlled processes might be supportive to inhibit drug-cue driven approach behaviors. Cognitive strategies of devaluating drug-related cues have been shown to enhance abstinence in smokers (Rose, 2006). Recently, Min et al. (2011) reported higher abstinence rates when heroin users underwent a relapse prevention program after detoxification. The training of cognitive strategies to reduce the motivational impact of heroin-related cues should therefore be considered as a crucial part of relapse prevention programs.

The present study has some limitations. First, the sample comprised mainly men, who differ from women in their subjective and physiological reactions to heroin-related cues as it has been previously shown (Yu et al., 2007). The results should therefore be replicated in women to be generalized. Second, even though urine toxicology confirmed current abstinence in former heroin users, abstinence duration measures rely on self-report and cannot be verified objectively, which is, however, an inevitable constraint. Third, in Study I, methadone treatment has been reduced two days before T2, which might have influenced emotional reactivity. However, Savvas et al. (2012), showed that effects of methadone on emotional processing were most prominent at peak plasma concentrations (around 3h post-dose) but 24h later no differences in emotional reactivity were found between controls and opioid-dependent subjects. Thus, we considered the residual influence of methadone on emotional reactivity to be very low at T2. Fourth, the sample size is moderate. However, this study (Study I) is the first longitudinal study employing an electrophysiological approach to measure the incentive value of drug-related cues. Moreover, it is also the first study comparing current and long-term abstinent heroin users with controls (Study II). Thus, despite of the moderate sample size

this study provides unique insights regarding the stability of incentive values of heroin-related cues in heroin users. Finally, in Study I, it cannot be ruled out that test-retest effects influenced the results of the second assessment, although the startle reaction is involuntary and assessments were separated by two weeks.

Conclusions

Heroin-related cues showing the end of a heroin injection scene are appetitive in current heroin-dependent subjects, and these cues retain their appetitive valence even after a detoxification therapy of 14 days as well as after long-term abstinence for more than one year. These findings indicate that chronic heroin use leads to stable and long-lasting adaptations of neural pathways resulting in a high risk for relapse over a long time period. However, cognitive control processes seem to play a crucial role to maintain abstinence despite increased reactivity to heroin-related cues because explicit and implicit reactions are dissociated in long-term abstinent heroin users.

Further studies are needed to examine whether the implicit appetitive response to heroin-related cues declines after prolonged abstinence for more than one year and whether this can be influenced by special treatment programs. Further, interventions which strengthen the devaluation of drug cues and apply stimulus control techniques should be part of relapse prevention programs.

Declaration of Interest

Experimental design, data acquisition, statistical analyses, and interpretation of the results were conducted without input from any pharmaceutical company. All authors report no biomedical financial interests or potential conflicts of interest with respect to this study.

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Authors Contributions

KHP, MW, and BBQ contributed to data analysis and writing of the manuscript. BBQ, KH, and MW contributed to the study design and development of the startle paradigm. AKR contributed to the writing of the manuscript. CS contributed to study design, data acquisition, and writing the manuscript. JN contributed to data acquisition and writing of the manuscript. IF and NP contributed to preprocessing of the data, data analysis, and writing the manuscript. PEF recruited and treated the patients and contributed to the writing of the manuscript.

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Table 1 Demographic characteristics (mean and standard deviation [SD]) of controls, current heroin users and former heroin users. Statistics were carried out for Study I (current heroin users vs. healthy controls) and Study II (current heroin users vs. former heroin users vs. healthy controls).

	Healthy controls (n=15)	Current heroin-dependent (n=15)	Former heroin-dependent (n=14)	**Study I** Current heroin dependent vs. healthy controls			**Study II** Current heroin dependent vs. former heroin dependent vs. healthy controls		
Study	I & II	I & II	II	Value	df/dferr	p	Value	df/dferr	p
Male participants	12	14	9	$\chi2 = 1.154$	1	0.283	$\chi2 = 3.758$	2	0.153
Age	29.53 (6.66)	30.93 (7.09)	35.57 (5.79)	F = 0.311	1/29	0.582	F = 3.339	2/41	0.045*
Years of education	10.87 (2.13)	9.20 (0.56)	10.36 (1.36)	F = 8.562	1/29	0.007*	F = 4.795	2/41	0.013*
Verbal IQ	95.70 (10.97)	92.36 (7.08)	102.00 (5.97)	F = 0.935	1/29	0.348	F = 4.800	2/41	0.014*
SCL-90-R sum score	32.73 (26.11)	61.87 (26.41)	35.14 (26.33)	F = 9.228	1/29	0.005*	F = 5.628	2/41	0.007*
Craving for heroin (0-80)	-	31.07 (11.03)	24.78 (7.51)	-	-	-	F = 3.167	1/28	0.086
Withdrawal symptoms (0-115)	-	41.93 (12.81)	24.79 (7.51)	-	-	-	F = 8.133	1/28	0.008*

[a]ANOVA or Chi^2-test within Study II; *indicates significant differences between groups

Table 2 Duration and quantity of drug use of controls, current heroin users and former heroin users (mean and standard deviation [SD]).

Study	Healthy controls (n=15) I & II	Current heroin-dependent (n=15) I & II	Former heroin-dependent (n=14) II
Heroin			
Days of use[a]	-	28.00 (4.14)	-
Years of use	-	6.27 (3.77)	8.57 (7.35)
Nicotine			
Cigarettes per day (CPD)	19.60 (8.57)	20.17 (10.87)	16.62 (6.20)
Years of use	10.47 (5.37)	16.40 (3.05)	19.08 (5.68)
Alcohol			
Days of use[a]	6.40 (7.99)	6.80 (9.74)	3.50 (5.95)
Years of use	0.87 (2.64)	3.27 (3.73)	5.64 (6.92)
Benzodiazepines			
Days of use[a]	-	0.93 (1.94)	-
Years of use	-	1.00 (2.48)	0.30 (0.61)
Cocaine			
Days of use[a]	-	3.80 (8.18)	-
Years of use	-	0.67 (1.23)	4.54 (7.26)
Amphetamines			
Days of use[a]	-	0.4 (1.30)	-
Years of use	-	0.53 (1.30)	0.64 (1.34)
Cannabis			
Days of use[a]	2.73 (5.60)	3.13 (7.61)	2.43 (7.98)
Years of use	1.20 (2.81)	5.80 (6.09)	6.50 (8.20)

[a]Days of use refers to the number of days on which the drug was used during the last 30 days

Figure legends

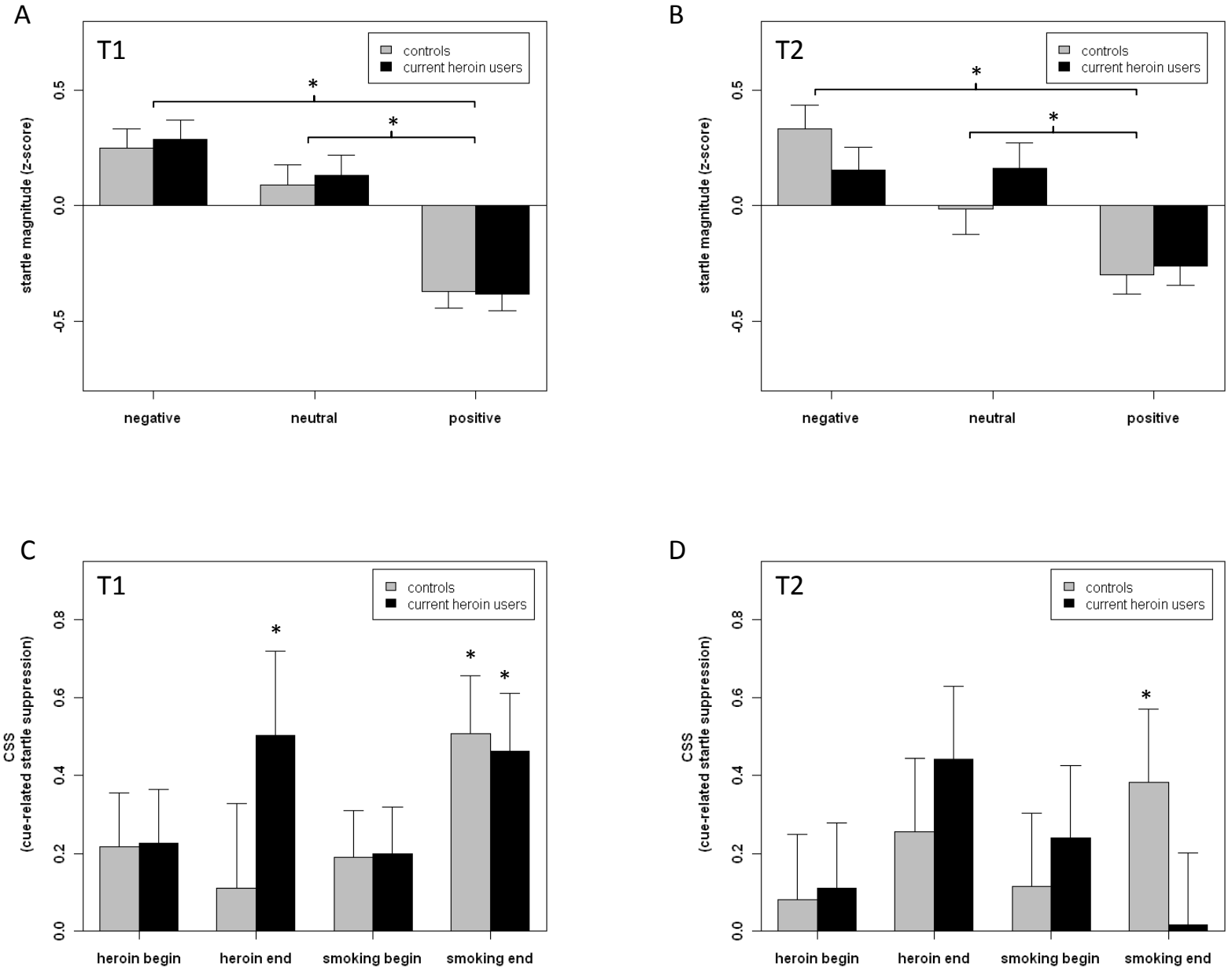
Fig. 1 – Mean standardized startle amplitudes during the presentation of control pictures on T1 (A) and T2 (B) and mean CSS during the presentation of drug-related cues on T1(C) and T2 (D). CSS was computed by subtracting the mean standardized startle amplitudes for drug-related pictures from mean standardized startle amplitudes for neutral pictures. Means are adjusted for age. Error bars refer to SEM. *indicates significant ($p<0.05$) differences between conditions (A&B) and significant ($p<0.05$) differences between standardized startle amplitudes in neutral and drug-related scenes (C&D).

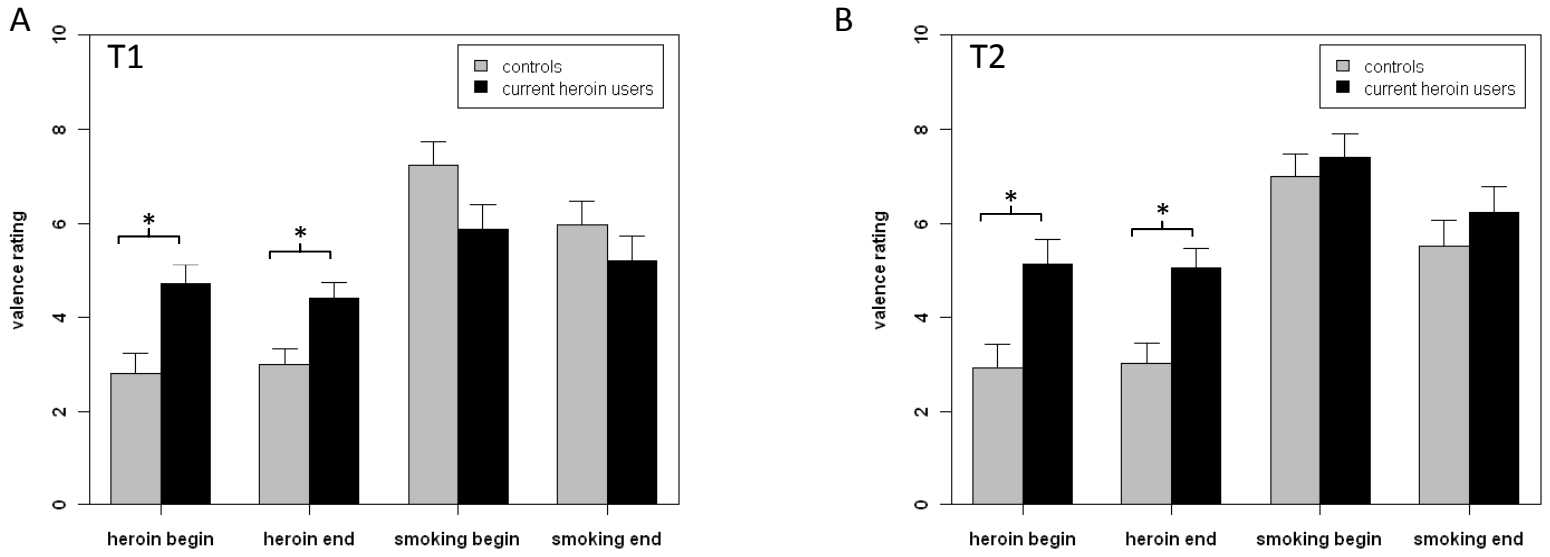
Fig. 2 – Mean valence ratings during drug-related pictures onT1 (A) and T2 (B) for controls (n=15) and current heroin users (n=15). Means are adjusted for age. Error bars refer to SEM. *indicates significant ($p<0.05$) differences between groups.

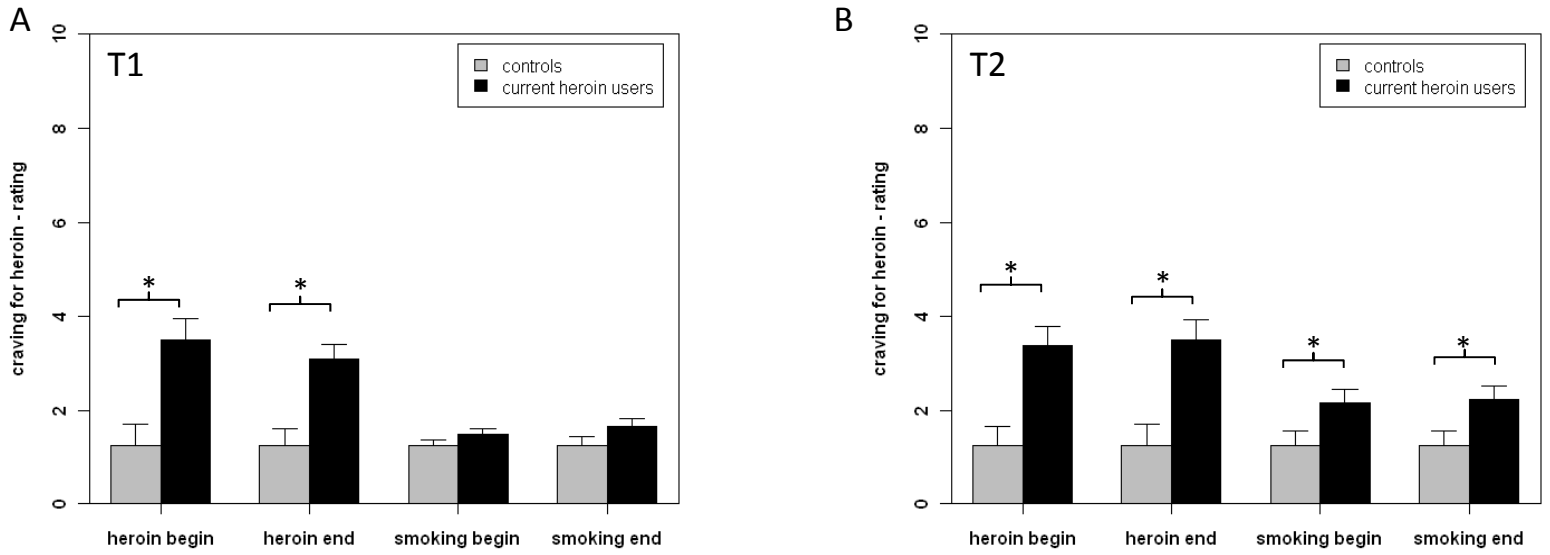
Fig. 3 – Mean craving for heroin-ratings during drug-related pictures onT1 (A) and T2 (B) for controls (n=15) and current heroin users (n=15). Means are adjusted for age. Error bars refer to SEM. *indicates significant ($p<0.05$) differences between groups.

Fig. 4 – Mean standardized startle amplitudes during the presentation of control pictures (A) and mean CSS during the presentation of drug-related cues (B) for controls (n=15), current heroin users (n=15) and former heroin users (n=14). CSS was computed by subtracting the mean standardized startle amplitudes for drug-related pictures from mean standardized startle amplitudes for neutral pictures. Means are adjusted for age. Error bars refer to SEM. Controls and current heroin users in Study 2 are the same as in Study 1. *indicates significant ($p<0.05$) differences between conditions (A) and significant ($p<0.05$) differences between standardized startle amplitudes in neutral and drug-related scenes (B).

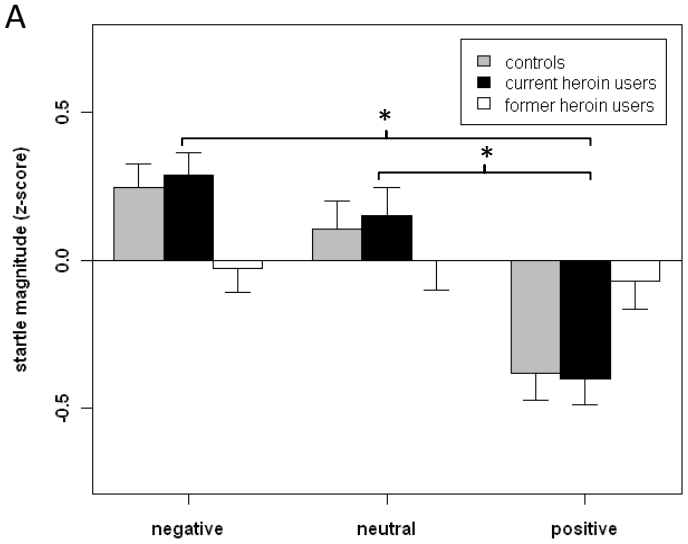
Fig. 5 – Mean valence (A) and craving for heroin (B) ratings during drug-related pictures for controls (n=15), current heroin users (n=15), and former heroin users (n=14). Means are adjusted for age. Error bars refer to SEM. Controls and current heroin users in Study 2 are the same as in Study 1. *indicates significant ($p<0.05$) differences between groups.


A
T1
controls
current heroin users
startle magnitude (z-score)
0.5
0.0
-0.5
*
*
negative
neutral
positive
B
T2
controls
current heroin users
startle magnitude (z-score)
0.5
0.0
-0.5
*
*
negative
neutral
positive
C
T1
controls
current heroin users
CSS
(cue-related startle suppression)
0.0
0.2
0.4
0.6
0.8
*
*
*
heroin begin
heroin end
smoking begin
smoking end
D
T2
controls
current heroin users
CSS
(cue-related startle suppression)
0.0
0.2
0.4
0.6
0.8
*
heroin begin
heroin end
smoking begin
smoking end

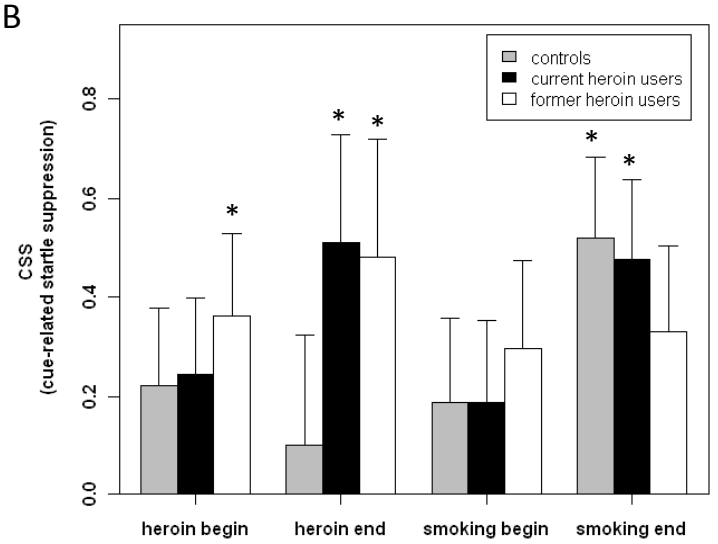

A
T1
controls
current heroin users
valence rating
0
2
4
6
8
10
*
*
heroin begin
heroin end
smoking begin
smoking end
B
T2
controls
current heroin users
valence rating
0
2
4
6
8
10
*
*
heroin begin
heroin end
smoking begin
smoking end

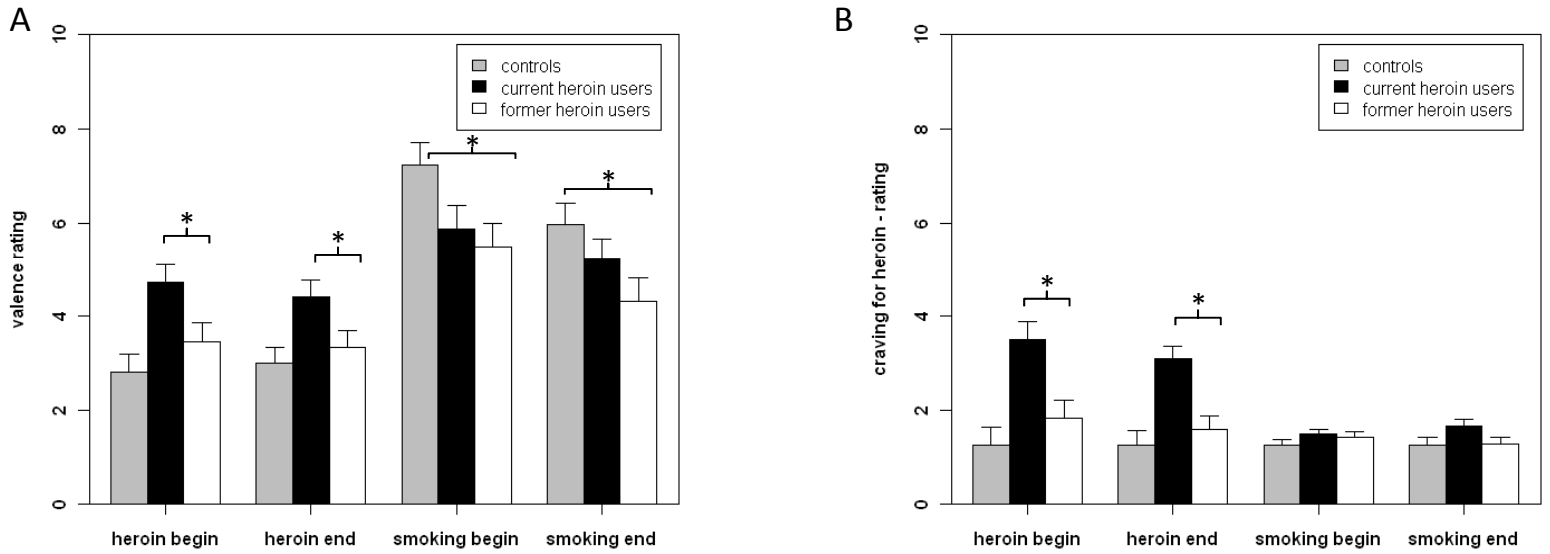

A
T1
controls
current heroin users
craving for heroin - rating
0
2
4
6
8
10
*
*
heroin begin
heroin end
smoking begin
smoking end
B
T2
controls
current heroin users
craving for heroin - rating
0
2
4
6
8
10
*
*
*
*
heroin begin
heroin end
smoking begin
smoking end

A


controls
current heroin users
former heroin users
startle magnitude (z-score)
0.5
0.0
-0.5
*
*
negative
neutral
positive

B


controls
current heroin users
former heroin users
CSS
(cue-related startle suppression)
0.0
0.2
0.4
0.6
0.8
*
*
*
*
*
*
heroin begin
heroin end
smoking begin
smoking end


A
valence rating
0
2
4
6
8
10
controls
current heroin users
former heroin users
*
*
*
*
heroin begin
heroin end
smoking begin
smoking end
B
craving for heroin - rating
0
2
4
6
8
10
controls
current heroin users
former heroin users
*
*
heroin begin
heroin end
smoking begin
smoking end

Supplementary Data

Sustained incentive value of heroin-related cues in short- and long-term abstinent heroin users

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Supplementary text

Data recording and reduction

The eye-blink component of the acoustic startle response was measured by an electromyographic (EMG) startle system (EMG-SR-Lab; San Diego Instruments, Inc., San Diego, CA). EMG activity was measured from the right *orbicularis oculi* muscle using two silver/silver chloride electrodes. A reference electrode was placed on the glabella. All electrode resistances were less than 10kΩ. EMG was recorded at a sampling rate of 1000Hz with a notch filter of 50Hz and a band-pass filter between 1 and 1000Hz from the onset of the acoustic startle stimulus for 250 ms.

Voluntary and spontaneous eye blinks were excluded from further analysis using the registration parameters described by Braff et al. (1992). The latency to startle response onset was defined by a shift of 2.28μV (six digital units) from the baseline value and occurring in a time window of 21–120ms after the acoustic startle stimulus. Response rejections were made both in case of onset-to-peak latencies >95ms and baseline shifts >34.2μV (>90 digital units). Additionally, startle responses were discarded if the amplitude was more than three standard deviations above the individual mean or if the amplitude was less than 25 digital units. The subject was taken out if there were less than two values per picture category after application of these criteria. Because the amplitude of the startle response underlies a habituation effect over trials (Bradley et al., 1993), we computed a regression analysis in order to correct our data against this effect. The decline of startle magnitude was best described by a logarithmic trend. Consequently, the raw data for every subject in the different trials were corrected for the logarithmic habituation trend. Finally, the available responses for the different picture categories were averaged to obtain the actual score.

Stimulus material

Stimulus material comprised 56 colored photographs, presented on a 12-inch monitor Notebook with a screen resolution of 600x800 pixels at 60–70cm in front of the participant. Sixteen self-taken pictures were arranged as eight pairs showing the beginning and end of a heroin injection scene (**Supplemental Material Figure S1**). Eight further pairs pictured the beginning and end of smoking a cigarette, which were taken from a previous study (Mucha et al., 1999, Experiment 2) (**Supplemental Material Figure S2**). Twenty-four control photographs were chosen from the IAPS (Lang et al., 1997), comprising eight pleasant

(8030,8080,8380,8370,4180/4490,4290/4510,4660,2840), eight neutral (78203,5534,6150, 7002,7030,7050,7190,7002) and eight negative scenes or objects (9410,3000,3010,3102, 3170,3150,3530,6230). The task was presented using the Experimental Run Time System (ERTS) software (Berisoft Cooperation, Frankfurt, Germany).

Data analysis

To analyze the affective reactivity in response to emotional control pictures (negative/neutral/positive) a 2*3*2 (time*picture category*group) mixed design ANCOVA was conducted in both studies. Startle reactivity in response to drug-related pictures (heroin begin/heroin end/smoking begin/smoking end) was analyzed by a 2*4*2 (time*picture category*group) mixed design ANCOVA. Explicit picture ratings were analyzed in the same way. In Study II, 3*3 (picture category*group) mixed design ANCOVAs were conducted for emotional control stimuli and 4*3 (picture category*group) ANCOVAS for drug-related pictures.

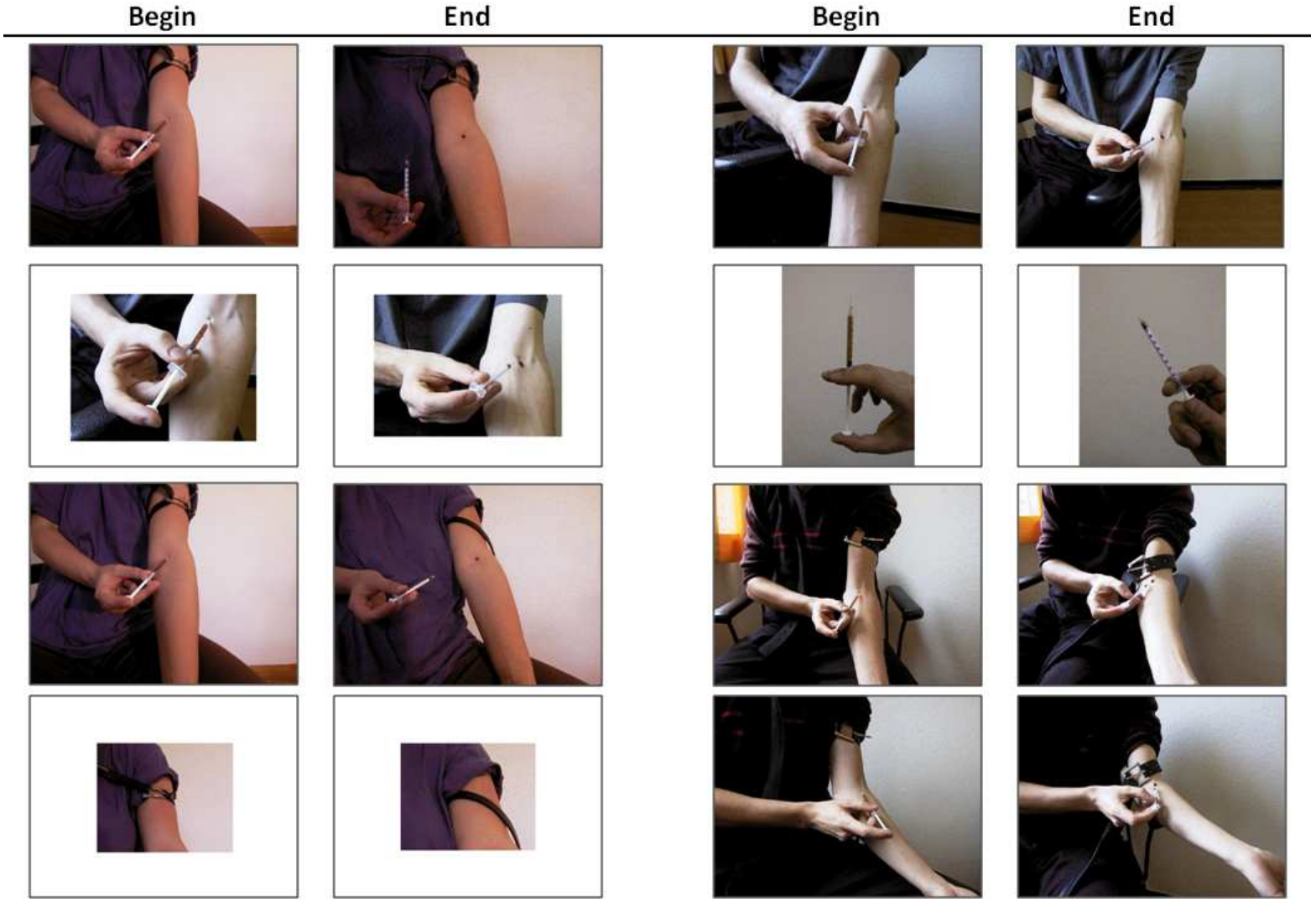

Fig. S1 – Heroin stimuli used in the affective startle paradigm. The sixteen self-taken pictures were produced as eight pairs showing the beginning and end of a heroin injection scene. During the paradigm they were presented together with neutral, positive, negative and smoking stimuli in a fixed randomized order.

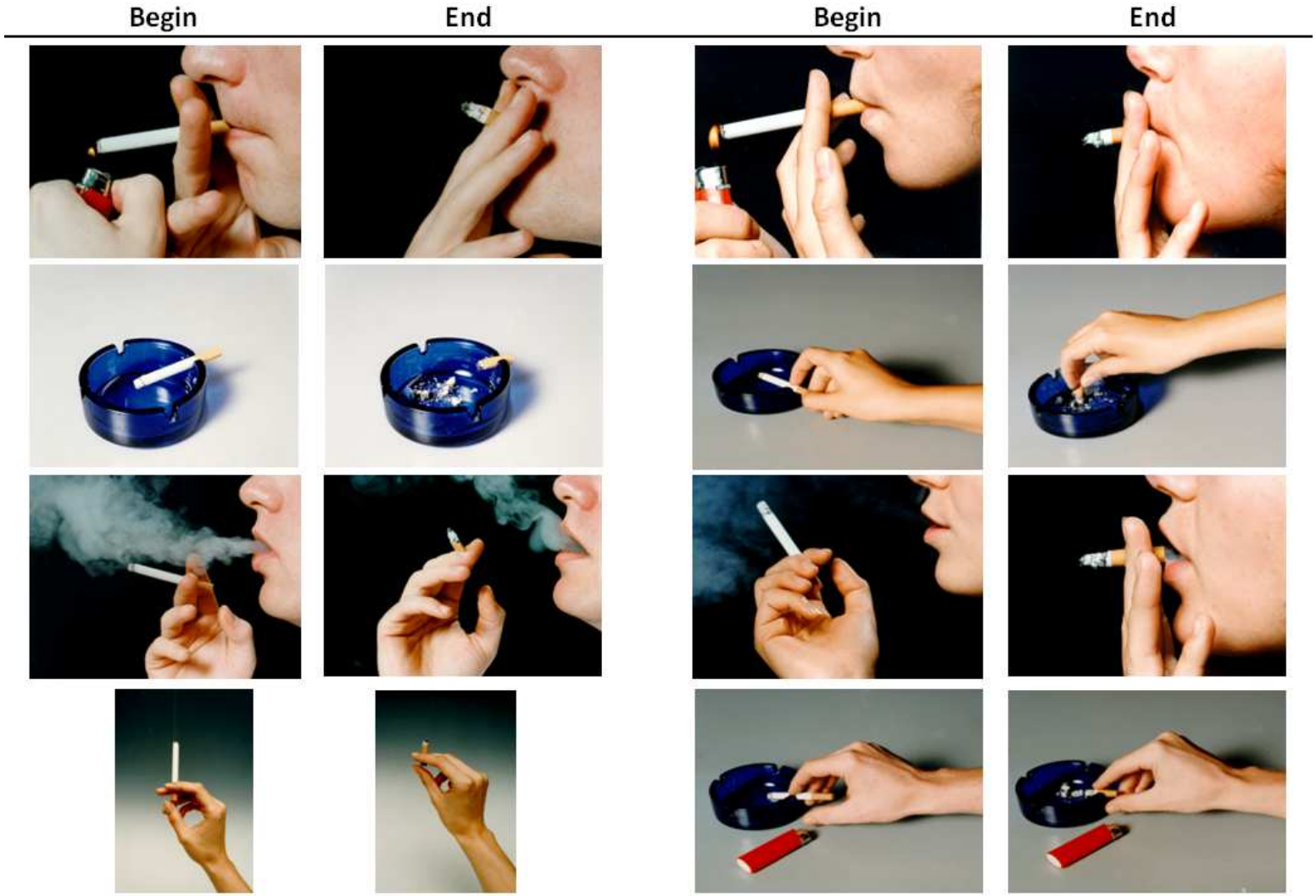

Fig. S2 – Smoking stimuli used in the affective startle paradigm. The sixteen self-taken pictures were produced as eight pairs showing the beginning and end of a smoking scene. Pictures were taken from a previous study (Mucha et al., 1999, Experiment 2). During the paradigm they were presented together with neutral, positive, negative and heroin stimuli in a fixed randomized order.

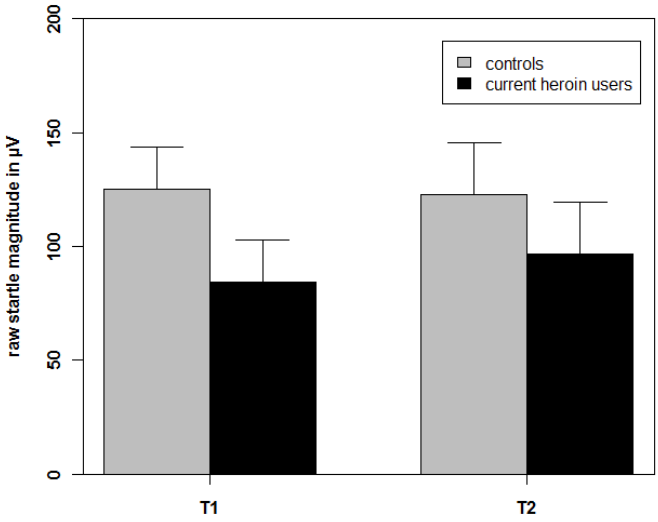

Fig. S3 - Mean raw startle magnitudes in µV in controls (n=15) and current heroin users (n=15) at T1 and T2. Error bars refer to SEM.

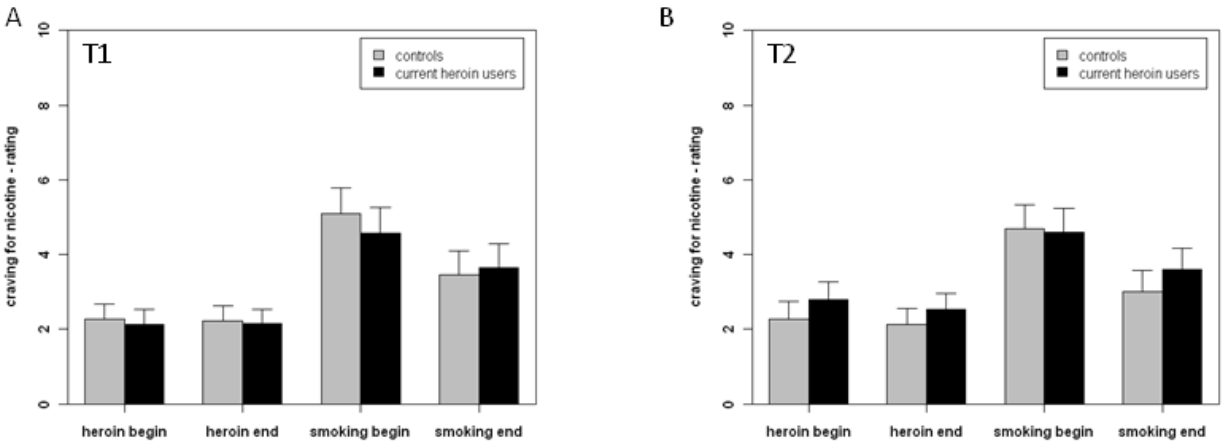

Fig. S4 – Mean craving for nicotine ratings during drug-related pictures on T1 (A) and T2 (B) for controls (n=15) and current heroin users (n=15). Means are adjusted for age. Error bars refer to SEM.

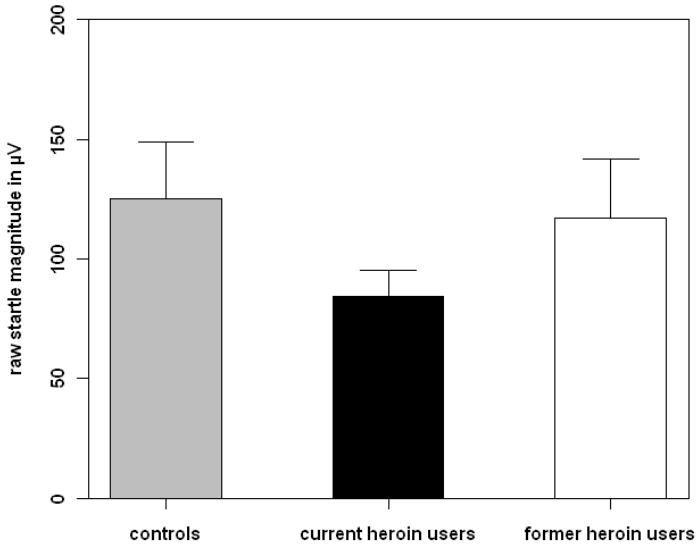

Fig S5 –Mean raw startle magnitudes in µV for controls (n=15), current heroin users (n=15) and former heroin users (n=14). Error bars refer to SEM.

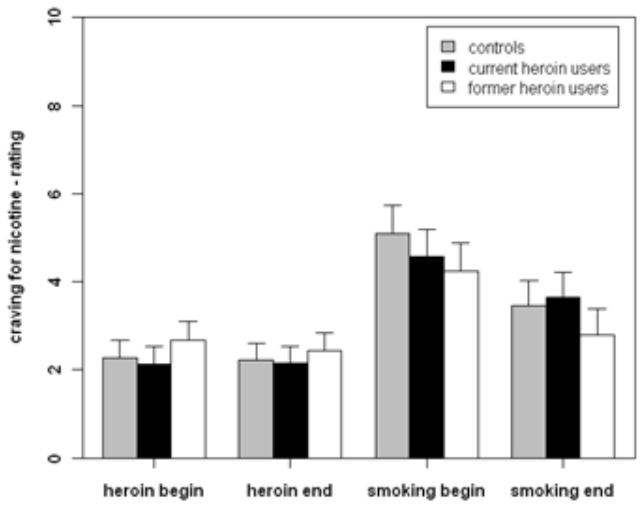

Fig. S6 - Mean craving for nicotine ratings during drug-related pictures for controls (n=15), current heroin users (n=15), and former heroin users (n=14). Means are adjusted for age. Error bars refer to SEM.

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